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Bcl-2 Inhibitors Sensitize Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand-Induced Apoptosis by Uncoupling of Mitochondrial Respiration in Human Leukemic CEM Cells

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ABSTRACT

Previous studies have shown that the lymphoblastic leukemia CEM cell line is resistant to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis because of a low expression of caspase-8. Bcl-2 inhibitors, BH3I-2′ and HA14-1, are small cell-permeable nonpeptide compounds, are able to induce apoptosis by mediating cytochrome *c* release, and also lead to dissipation of the mitochondrial membrane potential ($\Delta\Psi m$). This study aimed to use the Bcl-2 inhibitors to sensitize CEM cells to TRAIL-induced apoptosis by switching on the mitochondrial apoptotic pathway. We found that a low dose of BH3I-2′ or HA14-1, which did not induce cytochrome *c* release, greatly sensitized CEM cells to TRAIL-induced apoptosis. In a similar manner to the classical uncoupler carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), both BH3I-2′ and HA14-1 induced a reduction in $\Delta\Psi m$, a generation of reactive oxygen species (ROS), an increased mitochondrial respiration, and a decreased ATP synthesis. This uncoupling function of the Bcl-2 inhibitors was responsible for the synergy with TRAIL-induced apoptosis. CCCP *per se* did not induce apoptosis but again sensitized CEM cells to TRAIL-induced apoptosis by uncoupling mitochondrial respiration. The uncoupling effect facilitated TRAIL-induced Bax conformational change and cytochrome *c* release from mitochondria. Inhibition of caspases failed to block TRAIL-mediated cell death when mitochondrial respiration was uncoupled. We observed that BH3I-2′, HA14-1, or CCCP can overcome resistance to TRAIL-induced apoptosis in TRAIL-resistant cell lines, such as CEM, HL-60, and U937. Our results suggest that the uncoupling of mitochondrial respiration can sensitize leukemic cells to TRAIL-induced apoptosis. However, caspase activation *per se* does not represent an irreversible point of commitment to TRAIL-induced cell death when mitochondrial respiration is uncoupled.

INTRODUCTION

Apoptosis initiated by TRAIL is largely dependent on the cell-extrinsic signaling pathway, which involves death receptor engagement, the death-inducing signaling complex formation, proteolytic activation of the apical caspases, caspase-8 and -10, and consequently, activation of effector caspases such as caspase-3, -6, and -7 (1, 2). In certain types of cells, effector caspase activation requires amplification of death-inducing signaling complex signals by engagement of the cell-intrinsic pathway. A critical step in the cell-intrinsic pathway is the activation of Bax, leading to dissipation of the mitochondrial transmembrane potential ($\Delta\Psi m$) and cytochrome *c* release into the cytosol. This facilitates assembly of the Apaf-1 apoptosome with recruitment and activation of caspase-9 and subsequently the effector caspases (3). Multidomain proapoptotic members of the Bcl-2 family, such as Bax and Bak, are counterbalanced by the antiapoptotic family members Bcl-2 or Bcl-XL (4). BH3-only proteins, such as Bid, interact with proapoptotic Bcl-2 family members to augment their activity. Once cleaved by caspase-8 during treatment with TRAIL, Bid translocates to the mitochondria and activates Bax, thus providing a mechanism for cross-talk between the extrinsic and intrinsic apoptotic pathways (5, 6).

The requirement for Bax activation in TRAIL-induced apoptosis is cell type dependent (7–10). Early events triggered by TRAIL, such as death-inducing signaling complex formation, caspase-8 activation, and Bid cleavage were not dependent on Bax; however, mitochondrial depolarization, cytochrome *c* release, and activation of caspase-9 were prevented in Bax-deficient cells (9, 11). Thus, in these cells, the intrinsic pathway was required for TRAIL-mediated apoptosis, with Bax being essential for induction of the mitochondrial events.

BH3I-2′ and HA14-1 are small nonpeptidic organic compounds that interact with the surface pocket of Bcl-2 and can be used as cell-permeable agents to affect Bcl-2-regulated apoptotic pathways and are called inhibitors of Bcl-2 or BH3-mimetic compounds (12, 13). These small compounds not only induce cytochrome *c* release from mitochondria but also dissipate $\Delta\Psi m$ (12–14).

The mitochondria of healthy cells maintain an electrochemical gradient across the mitochondrial inner membrane (MIM) that is created by pumping protons from the matrix to the inter-membrane space of these organelles in conjugation with electron transport through the respiratory chain. The proton gradient and membrane potential are the proton-motive force that is used to drive ATP synthesis. Coupling of electron transport through the respiratory chain and ATP generation can be disrupted by some acidic aromatic substances such as carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) and 2,4-dinitrophenol. These so-called uncouplers of oxidative phosphorylation carry protons across the inner mitochondrial membrane. This specific attack of oxidative phosphorylation leads to a reduction of $\Delta\Psi m$, to the cessation of ATP generation in the mitochondrion, and to the collapse of the pH gradient by shuttling protons back across the membrane (15).

Alteration in mitochondrial function can change the sensitivity of tumor cells to apoptosis mediated by death receptors. Increase in mitochondrial respiration sensitizes leukemic cells to tumor necrosis factor-mediated apoptosis (16). Depletion in mitochondrial DNA renders tumor cells resistant to apoptosis induced by TRAIL (17). The uncoupler CCCP can enhance the Fas death signal, although CCCP alone does not have an apoptotic effect (18). However, the precise mechanism by which the mitochondrial function contributes to death receptor-mediated apoptosis is still unclear.

In this study, we used BH3I-2′ or HA14-1 as a sensitizer for overcoming the resistance of leukemic cells to TRAIL-induced apoptosis. It was found that both BH3I-2′ and HA14-1 showed an uncoupling effect on the oxidative phosphorylation when they were used at the concentrations that could not induce cytochrome *c* release and apoptosis. CCCP, which does not induce apoptosis, also showed a large synergistic effect on TRAIL-induced apoptosis in leukemic cells. Our data showed that the synergistic effect of uncoupling agents on TRAIL-induced apoptosis is via the intrinsic apoptotic pathway

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Note: J-H. Hao and M. Yu contributed equally to this work.

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